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Mapping of two loci conferring resistance to wheat stem rust pathogen races TTKSK (Ug99) and TRTTF in the elite hard red spring wheat line SD4279

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Abstract Since its identification in the late 1990s, the stem rust pathogen (*Puccinia graminis.* f. sp. *tritic*i (*Pgt*)) strain Ug99 (race TTKSK) has represented a worldwide wheat production threat due to its ability to overcome most of the resistance genes present in commercial cultivars. In order to address this challenge, resistance genes in wheat cultivars as well as in wild relatives have been identified. However, stem rust resistance breeding is facing a new challenge with the recent discovery in Ethiopia of a new race of *Pgt* (TRTTF) capable of defeating *Sr13*, *SrTmp*, and $Sr1R^{Amigo}$ genes that conferred resistance to the Ug99 race group. As part of an ongoing screening process at USDA-ARS Cereal Disease Laboratory, SD4279, an elite line from the hard red spring wheat breeding program at South Dakota State University, was found to be resistant to both races TTKSK and TRTTF. The objectives posed in this research were (1) to characterize the genetics of resistance to stem rust in SD4279 and (2) to identify molecular markers linked to race TTKSK (Ug99) and TRTTF resistance in SD4279. A mapping population composed of 92 $F_{2:3}$ families was evaluated for resistance to TTKSK and TRTTF. A single-gene conferring resistance to TTKSK, likely *Sr9h*, was mapped on chromosome arm 2BL. Also, a single gene was located on chromosome arm 6AS conferring resistance to TRTTF. Based on the type of reaction and map location, we postulate that the 6AS resistance gene is *Sr8a* which has not been mapped previously using DNA markers.

Keywords *Puccinia graminis.* f. sp. *tritic*i · Ug99 · TRTTF · Resistance · Molecular markers

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Introduction

For the last several decades, worldwide wheat (*Triticum aestivum* L.) production was largely protected from stem rust (caused by *Puccinia graminis.* Pers.:Pers. f. sp. *tritic*i Eriks. and E. Henn.) because of genetic resistance conferred by stacking R genes (Singh et al. 2011). However, in 1999 this paradigm changed with the discovery in Uganda of a new *P. graminis* race, TTKSK, also known as Ug99. This new race was characterized as virulent to the majority of *Sr*

genes, including the widely used *Sr31* (Pretorius et al. 2000).

Ug99 has shown the ability to rapidly mutate into new virulent variants. Eight TTKSK variants have been reported in Africa and Asia, and only 10 % of the wheat varieties planted around the world are resistant to the current Ug99 race group (Singh et al. 2011; Visser et al. 2011; Pretorius et al. 2012). US breeding programs rely primarily upon *Sr24* and *Sr36* for resistance to race TTKSK (Jin and Singh 2006). Unfortunately, two Ug99 variants, races TTKST and TTTSK discovered in Kenya in 2006 and 2007, were found to be virulent to *Sr24* and *Sr36*, respectively (Jin et al. 2008, 2009), reducing the resistance gene pool and increasing the alarm of a possible global wheat production risk.

In order to face the challenge of Ug99 and its ability to rapidly mutate and overcome resistance, breeders are using wild wheat relatives as sources of stem rust resistance genes. From *T. monoccocum* L., *Sr21*, *Sr22*, and *Sr35* have been found to be resistant to race TTKSK (Rouse and Jin 2011). *Sr35* is resistant to TTKSK, but susceptible to other rust races and therefore needs to be used in combination with other *Sr* genes (Saintenac et al. 2013).

Similarly, *Sr32*, *Sr39*, *Sr47*, *SrAes1t*, *SrAes7t*, transferred from *Aegilops speltoides* (Klindworth et al. 2012; Mago et al. 2013) and *Sr44*, derived from a translocation with *Thinopyrum intermedium* (Liu et al. 2013), are resistant to TTKSK and two of its derivatives (TTKST and TTTSK).

Aegilops tauschii (D genome) has been a source of genes conferring resistance to Ug99 such as *Sr33*, *Sr45*, *Sr46*, *SrTA1662*, *SrTA10171*, and *SrTA10187* (Rouse et al. 2011a; Olson et al. 2013a, b). Stem rust resistance genes *Sr13*, *Sr25*, *Sr26*, *Sr27*, *Sr37*, *Sr40*, *Sr51*, *Sr52*, *Sr53*, and $Sr1RS^{Amigo}$ from *T. turgidum*, *T. elongatum*, *Secale cereal*, *T. timopheevi*, and *T. araraticum* are considered as resistant or moderately resistant to Ug99 in seedlings and adult plants (Jin et al. 2007). Genes *Sr51*, *Sr52*, and *Sr53* derived from *A. searsii*, *Dasypyrum villosum*, and *A. geniculata* (Liu et al. 2011a, b; Qi et al. 2011) are resistant as seedlings to race TTKSK.

The utilization of introgresed *Sr* genes in commercial cultivars has been limited due to linkage drag of non-favorable or suppressed gene effects associated with alien chromatin (Liu et al. 2013). Only few genes from common wheat (*T. eastivum*) have been reported as resistant to Ug99. Those genes are: *Sr9h*, *Sr28*, *Sr29, Sr42*, *Sr48, Sr57(*Lr34), *SrCad*, *SrTmp*, *SrSha7*, *SrHuw234*, and *SrND643* (Jin and Singh 2006; Singh et al. 2008; Hiebert et al. 2011; Singh et al. 2011; Ghazvini et al. 2012; Rouse et al. 2012, 2014; Singh et al. 2013).

Recently, a *Pgt* strain different from the Ug99 race group was discovered in Yemen and identified as race TRTTF. Genes *Sr13*, *SrTmp*, and $Sr1RS^{Amigo}$ which are resistant to the Ug99 race group show susceptibility to TRTTF. The genes *Sr8a*, *Sr22*, *Sr24*, *Sr26*, *Sr27*, *Sr31*, *Sr32*, *Sr33*, *Sr35*, *Sr39*, *Sr40*, *Sr46*, *Sr47*, *Sr50*, and *SrSatu* were classified as resistant to race TRTTF (Olivera et al. 2012).

Of the Ug99-effective *Sr* genes mapped, only *Sr22* and *Sr26* provide resistance to all stem rust races (Singh et al. 2011). Since *P. graminis* has already demonstrated its ability to mutate, more sources of resistance are needed, especially those derived from the primary gene pool. SD4279, an elite line from the hard red spring wheat (HRSW) breeding program at SDSU was found to be resistant to races TTKSK and TRTTF. The fact that SD4279 is resistant to both races makes it an ideal potential parental line for the hard red spring wheat breeding programs in North America. In order to answer the interrogatives about the sources of resistance present in SD4279, our objectives were to (1) characterize the genetics of resistance to stem rust in SD4279 and (2) identify molecular markers linked to race TTKSK (Ug99) and TRTTF resistance in SD4279.

Materials and methods

Mapping population

A bi-parental mapping population consisting of 92 F_2 individuals corresponding to 184 gametes was derived from a cross of SD4279 × "Brick." Both parents originate from the hard red spring wheat (HRSW) breeding program at South Dakota State University. Brick is a variety derived from a three-way cross with pedigree ND2897/SD3219//SD3414 (Glover et al. 2010). ND2897 is an experimental line from North Dakota State University HRSW breeding program. SD3219 and SD3414 are two experimental lines from the South Dakota State University HRSW breeding program. Brick was released in 2009 with high yield

and resistance to *Fusarium* head blight as well as moderate resistance to leaf rust, but susceptible to TTKSK and TRTTF (Glover et al. 2010). SD4279 is an elite line with pedigree NDSW0601/00S0219-10W. Both parents of SD4279 are unreleased experimental breeding lines. NDSW0601 originates from the NDSU hard white and specialty spring wheat breeding program, and 00S0219-10W is from the AgriPro/Syngenta hard red spring wheat breeding program. The 92 F_2 plants were grown in the greenhouse to obtain DNA for genotyping and F_3 seed.

Disease assessment

Seedling reaction to *P. graminis* f. sp. *tritici* races TTKSK (Isolate 04KEN156/04) and TRTTF (Isolate 06YEM34) was evaluated in independent greenhouse experiments using 20–24 seedlings for each of the 92 $F_{2:3}$ families at the USDA-ARS Cereal Disease Laboratory in St. Paul, Minnesota, in 2012. The seedlings were inoculated as stated in the procedure described by Rouse et al. (2011b) at 7–9 days after sowing with a spore concentration of 3–5 mg ml^{-1}. Infection types (ITs) were recorded 14 days post-inoculation on a 0–4 scale (Stakman et al. 1962) where ITs from 0 to 2 were considered as resistant and ITs 3–4 as susceptible. When a family produced only seedlings with 0, 1, and 2 ratings, the parent F_2 plant was considered homozygous for the resistant allele of a resistance gene; similarly when a family produced only seedlings with 3 and 4 ratings, the parent F_2 plant was considered homozygous for the susceptible allele. Families with resistant and susceptible seedlings were considered to be derived from a heterozygous parent F_2 plant. A Chi-square test was conducted for goodness of fit between the observed and expected segregation ratio (1:2:1) according to Mendelian heritability for a single gene in the F_2 population.

Genotyping and linkage analysis

DNA was extracted from leaf tissue of 5-week-old F_2 plants following a modified phenol–chloroform extraction method (Karakousis and Langridge 2003).The amount of DNA extracted was quantified, and the quality was verified in 1 % agarose gel. DNA samples from 92 F_2 individuals and two parents were run on a custom 9K i Select SNP Beadchip Array (Cavanagh et al. 2013) in the USDA-ARS Biosciences Research Laboratory in Fargo, North Dakota. Base calling was done using Illumina GenomeStudio version 2011.1 software (Illumina Inc, San Diego, CA). SNP allele clustering was confirmed through visual examination and manually curated when the clusters were not clearly separated. Monomorphic markers or SNPs with parents clustered as heterozygous were discarded.

Additionally, parents were genotyped with 179 SSR markers chosen across the 21 wheat chromosomes based on their proximity to the centromere according to a consensus map (Somers et al. 2004). The libraries used for microsatellites were WMC, GWM, BARC, CFD, and GDM (74, 57, 32, 15, and 1 markers from each library, respectively). Microsatellites were amplified in PCR containing 50 ng of DNA template, 1 × buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 μM of forward and reverse primer and 1U of Taq Polymerase. The amplification was carried out 45 times in a thermo cycler with an annealing temperature of 51 or 60 °C (according to the marker specification) for 1 min. The fragments amplified were loaded in 6 % non-denaturing polyacrylamide gel with ethidium bromide and separated through electrophoresis. Polymorphic markers were used to genotype the F_2 population. The resistance classifications based on race TTKSK and TRTTF reactions were entered in the linkage analysis as two loci, one for each race. Loci were grouped and ordered with JoinMap4 (Van Ooijen 2006). Grouping was done with an independence LOD threshold starting at 3.0 and ending at 30.0. A regression mapping algorithm and Haldane's function were utilized to order the markers. A previous published SNP consensus map (Cavanagh et al. 2013) was used to identify the chromosomes corresponding to the linkage groups obtained from the Brick/SD4279 mapping population used in this study.

In order to confirm the mapped location of the qualitative stem rust resistance loci, a QTL mapping analysis was conducted. Quantitative values for each line were obtained by multiplying the number of plants in each infection type group within the line, by the numeric value of the group. The line average value was used to carry out a composite interval mapping (CIM) analysis for each trait in Windows QTL Cartographer 2.5 (Wang et al. 2012). The significance threshold value was set up through one thousand permutations, and five markers were used as cofactors

to control the genetic background. The cofactor markers were selected via forward–backward regression method with a window size of 10 cM. The effect of each resistance QTL, as well as their interaction over TTKSK and TRTTF seedling reaction, was assessed using R Stats 3.1.1 software package (R Core Team 2014) through a multivariate analysis of variance (MANOVA) using Pillai's test.

Results

SD4279 displayed resistant IT 2 to race TTKSK, whereas Brick displayed susceptible IT 3+. A total of 21 families were resistant (ITs 2 to 2+3) to race TTKSK; 47 were heterozygous (both resistant ITs 2 to 2+3 and susceptible IT 3+), and 23 $F_{2:3}$ families were susceptible with all plants from these families exhibiting susceptible IT 3+. Resistance to race TRTTF segregated 14 (resistant):51(heterozygous):27 (susceptible). The Chi-square test between the observed and expected infection types according to a single-gene segregation ratio 1:2:1 was 0.19 and 4.76 for TTKSK and TRTTF. Both were non-significant (p value >0.05), indicating that the resistance to both races segregated following single-gene Mendelian inheritance (Fig. 1).

A total of nine SSR markers and 1,114 polymorphic SNPs were mapped into twenty-five linkage groups with a LOD score ranging from 4.0 to 30.0. One or three linkage groups were assigned to 15 chromosomes using the consensus map reported by Cavanagh et al. (2013). Chromosomes 1D, 2D, 3D, 4D, 5D, and 6D had no linkage group assigned to them. The percentage of SNPs out of our linkage map matching the consensus map by Cavanagh et al. (2013) was

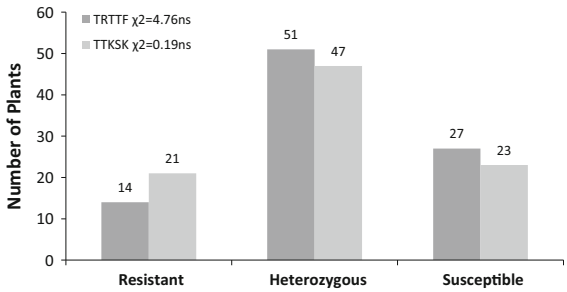

Fig. 1 Infection segregation of 92 DH lines to TRTTF and TTKSK races

92.5 %. The remaining SNPs were not localized in the consensus map (4.5 %), or their chromosomal assignation was discrepant (3.0 %). Chromosome 5B had the highest coverage with 148 SNPs distributed in two linkage groups (Supplemental Table 1).

The race TTKSK resistance gene was located at 48.6 cM in linkage group 5, which was formed by 87 SNPS and four SSR markers joined together with a LOD score of 11.0 and total length of 48.6 cM. Also, group 7 corresponded to chromosome 2B (Fig. 2). CFD73 was the nearest marker to the resistance gene as well as the SNPs 5789 (wsnp_JD_c11975_12326445) and 2318 (wsnp_Ex_c18503_27349536) with distances between them of 3.82, 5.76, and 5.89 cM, respectively (Table 1). In the consensus map, the resistance gene is located at around 175.0 cM from the distal part of the short arm of chromosome 2B.

The race TRTTF resistance gene was located terminal (0.0 cM) on the linkage group 25, which together with group 21 aligned to chromosome 6A (Fig. 3). Linkage group 25 is composed of eight SNPs plus the resistance locus with a total length of 8.39 cM with a LOD score of 18.0. The nearest markers to the resistance gene were the SNPs 7007 (wsnp_Ku_c39334_47795461) and 7913 (wsnp_Ra_c3996_7334169) located at 1.83 cM, followed by 705 (wsnp_CAP11_c2142_1128735) and 1033 (wsnp_CAP7_c1339_673581) at 7.25 cM (Table 1). Even though the gene is located at the edge in linkage group 25, the alignment to the consensus map published by Cavanagh et al. (2013) suggest that this locus is approximately 6 cM from the distal part of the short arm on the chromosome 6A.

The QTL analysis via composite interval mapping for TTKSK detected two QTLs with LOD scores superior to 3.5 which were considered as the threshold value set up by one thousand permutations. The first of the QTLs (Fig. 2) was located on group 5 (chromosome 2B) at 0.0001 cM apart from where the TTKSK gene was placed when it was considered as a single Mendelian inheritance gene. The LOD score was 48.8, and the trait accounted for 84.1 % of the variance with an additive value of 0.48 and a dominance value of −0.09. The parent contributing to the increase in disease was Brick.

A second QTL for TTKSK was observed at 29.0 cM in group 11 which linked to chromosome 1A with a LOD score of 4.1 (figure not showed). This QTL explained 2.3 % of the variation. The nearest marker was wsnp_Ra_c16278_24893033 and an

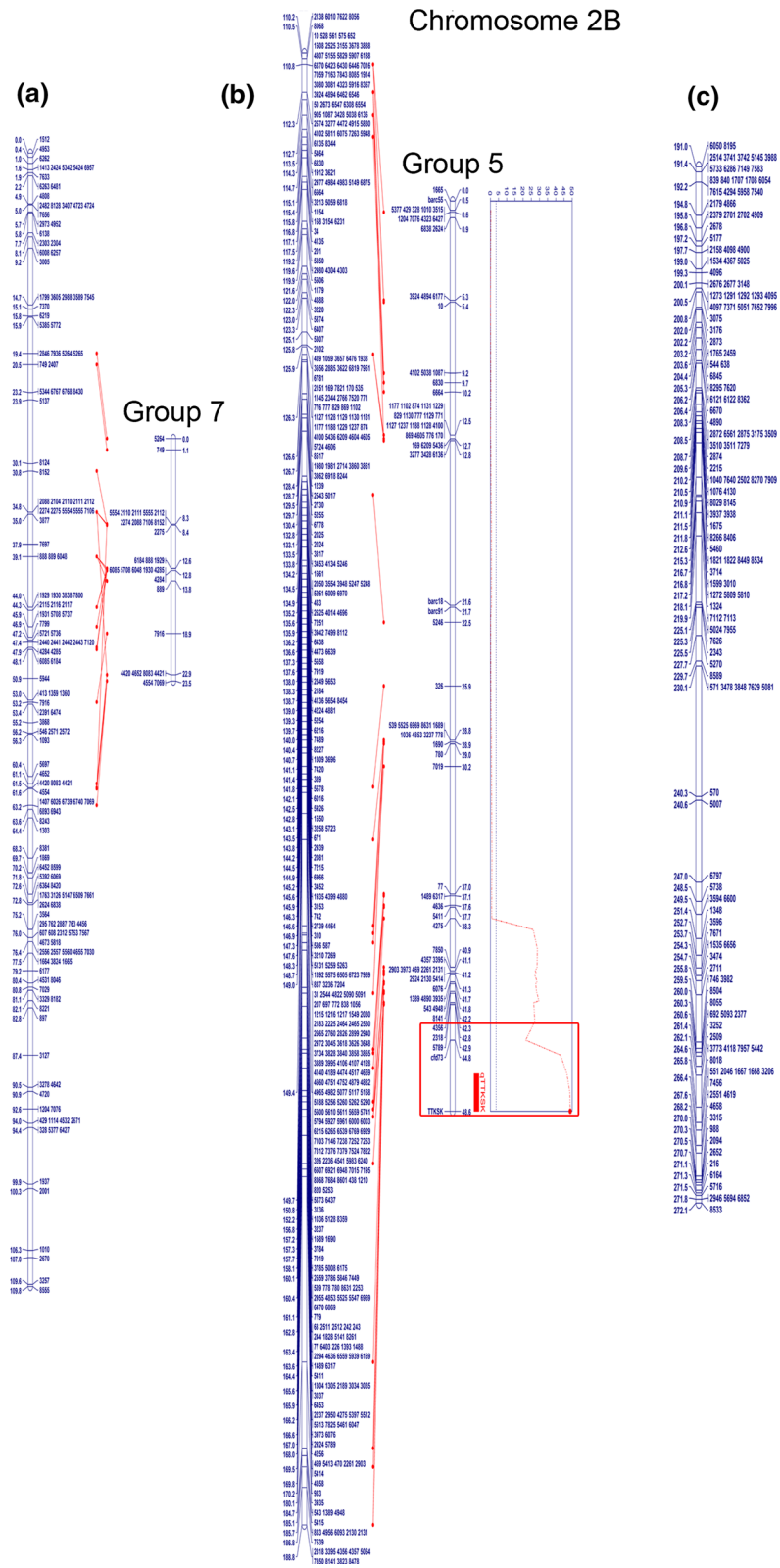

Fig. 2 Alignment of linkage groups 5 and 7 on chromosome 2B according to the consensus map (**a**–**c**) published by Cavanagh et al. (2013). A QTL for resistance to race TTKSK mapped to group 5 with a LOD score of 48.8 (*red dot*). The threshold to declare a QTL is represented by a *dotted line*. The *red solid bar* represents a confidence interval of 2 LOD scores. (Color figure online)

Table 1 Group, chromosome, and position of TTKSK, TRTTF, and their neighbor markers

SNP ID	Marker/gene name	Linkage group	Chromosome	Position (cM)
_	TTKSK	5	2BL	48.64
_	CFD73	5	2BL	44.82
5789	wsnp_JD_c11975_12326445	5	2BL	42.88
2318	wsnp_Ex_c18503_27349536	5	2BL	42.75
_	TRTTF	25	6AS	0.00
7007	wsnp_Ku_c39334_47795461	25	6AS	1.83
7913	wsnp_Ra_c3996_7334169	25	6AS	1.83
705	wsnp_CAP11_c2142_1128735	25	6AS	7.25
1033	wsnp_CAP7_c1339_673581	25	6AS	7.25

additive value of 0.07 and dominance value of 0.02. Brick was the parent, contributing the susceptible allele, and SD4279 contributed the resistant allele. For TRTTF trait, only one QTL was detected via CIM on group 25 (chromosome 6A) at 0.0001 cM from where the gene was placed when it was considered as a single-gene Mendelian inheritance (Fig. 3). The LOD score was 52.3 (threshold value of 3.3 LOD) and explained 89.4 % of the variation with an additive value of 0.48 and dominance value of −0.05. All the QTLs, additive value and dominance value are shown in Table 2.

The MANOVA showed significant effect ($p \leq 0.05$) on loci TRTTF and TTKSK (0.04 and 5×10^{-6} p value, respectively), but a not significant effect in the interaction between both loci with a p value of 0.72.

Discussion

Based on the single-gene Mendelian inheritance segregation (1:2:1) and mapping, we determined that race TTKSK and TRTTF resistance in SD4279 is conferred by two single genes located on chromosome arms 2BL and 6AS, respectively. The position of the genes is supported by the alignment of the linkage groups in the Cavanagh et al. (2013) consensus map sharing 92.5 % of the polymorphic SNPs in the population studied.

Also, the qualitative mapping results were confirmed through a composite interval mapping analysis, showing that QTLs for each trait located within 0.0001 cM of the mapping of the seedling resistance as single qualitative genes. The variance that was explained for each QTL was 84.1 and 89.4 % with LOD scores of 48.8 and 52.3 for TTKSK and TRTTF, respectively. Although a second QTL was identified for TTKSK on chromosome 1A, it does account only for a small proportion of the variation (2.3 %). Therefore, the second QTL identified can be a minor effect QTL or even a spurious QTL due to the genetic unaccounted noise.

The gene conferring resistance to Ug99 was located approximately at 175.0 cM from the distal part of the short arm of chromosome 2B and 3.82 cM from the nearest marker CFD73. Also SNPs 5789 (wsnp_JD_c11975_12326445) and 2318 (wsnp_Ex_c18503_27349536) are 5.76 and 5.89 cM apart from the gene, respectively. Interestingly, *Sr9h* which is known to be resistant to race TTKSK has been mapped in the chromosome 2BL at 8.6 cM from CFD73 (Hiebert et al. 2010; Rouse et al. 2014) and yielded similar infection types compared to those observed in our $F_{2:3}$ families. Therefore, we hypothesize that *Sr9h* is the gene on chromosome 2BL conferring resistance to Ug99 in the Brick/SD4279 population.

Additionally, we found that SD4279 carries a gene resistant to race TRTTF located on the distal part of the short arm of chromosome 6A. So far, *Sr8a*, *Sr22*, *Sr24*, *Sr26*, *Sr27*, *Sr31*, *Sr32*, *Sr33*, *Sr35*, *Sr39*, *Sr40*, *Sr46*, *Sr47*, *Sr50*, and *SrSatu* have been reported to be resistant to race TRTTF (Olivera et al. 2012). Of all of them, only *Sr8a* had been located in the short arm of chromosome 6A (McIntosh 1972; Sears et al. 1957). *Sr8* was first reported conferring resistance to stem rust races 15B and 56 in the variety "Red Egyptian" (Knott and Anderson 1956) and later transferred through backcrosses to "Marquis" (Green et al. 1960). Through cytogenetic studies, McIntosh (1972) confirmed the localization of *Sr8* on the short arm of chromosome 6A. An allelic variant of *Sr8* was found in varieties "Barleta" "Benvenuto," "Klein

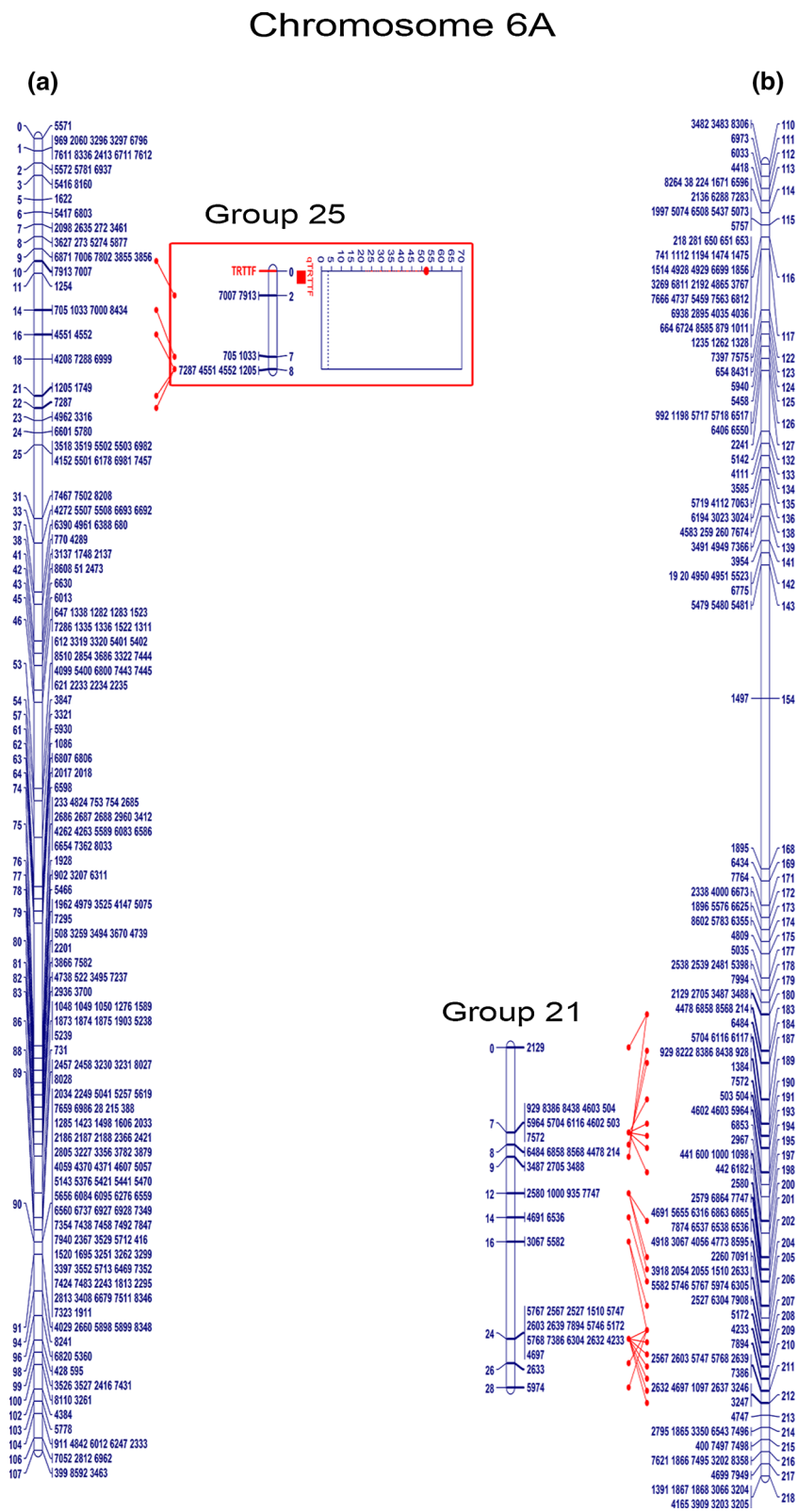

Fig. 3 Alignment of the linkage groups 21 and 25 on chromosome 6A according to the consensus map (**a**, **b**) published by Cavanagh et al. (2013). A QTL for resistance to race TRTTF mapped to group 25 with a LOD score of 52.3 (*red dot*). The threshold to declare a QTL is represented by a *dotted line*. The *red solid bar* represents a confidence interval of 2 LOD scores. (Color figure online)

Table 2 QTLs located trough composite interval mapping for TTKSK and TRTTF traits

Chromosome	Group	QTL	Position (cM)	Additive effect	Dominant effect	Susceptible parent
TTKSK						
2B	5	QTL1	48.6	0.48	−0.09	Brick
1A	11	QTL2	29.0	0.07	0.02	Brick
TRTTF						
6A	25	QTL1	0.0	0.48	−0.05	Brick

Titan," and "Klein Cometa" which was named as *Sr8b* and the one named before as *Sr8* was renamed as *Sr8a*. We hypothesize, according to the infection type and chromosomal localization, that the gene mapped in our population and *Sr8a* are the same stem rust-resistant gene. This is the first report of molecular markers linked to resistance gene that is likely *Sr8a*. SNP markers 7007 (wsnp_Ku_c39334_47795461) and 7913 (wsnp_Ra_c3996_7334169) were located within 1.83 cM of the resistance gene and could be used for validation experiments to determine the usefulness of these markers for marker-assisted selection of resistance to race TRTTF.

Also, in order to investigate the interaction effect between the two genes resistant to TTKSK and TRTTF, a multivariate analysis of variance (MANOVA) was conducted. The interaction effect between both genes was not significant at 0.05 p value, which indicates that the susceptibility to TTKSK is unconditioned by resistant or susceptibility to TRTTF and vice versa.

In this paper, we identify the SNPs 7007 (wsnp_Ku_c39334_47795461) and 7913 (wsnp_Ra_c3996_7334169) as the nearest markers to race TRTTF resistance at 1.83 cM apart followed by 705 (wsnp_CAP11_c2142_1128735) and 1033 (wsnp_CAP7_c1339_673581) at 7.25 cM. Gene *Sr9h* has been previously reported in older cultivars Webster and Gabo 56 (Hiebert et al. 2010; Rouse et al. 2014). We report year 2009 South Dakota State University breeding line SD4279 as likely possessing *Sr9h*. SD4279 is an ideal parental material to be used in wheat breeding programs to select for resistance to the Ug99 race group and to race TRTTF with little or no linkage drag.

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